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ALTERNATIVE METHODS FOR MEASURING VESICANT AGENT ACTION ON RABBIT SKIN

Peter Schmid, PhD, June R. Jaeger, BS, George J. Klain, PhD, John P. Hannon, PhD, Carol A. Bossone, MS, Michael C. Powanda, PhD

DIVISION OF CUTANEOUS HAZARDS

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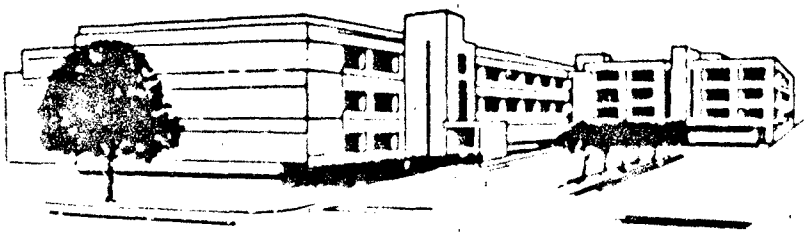

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Alternative methods for measuring vesicant agent action on rabbit skin--Schmid et al.

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Tissue damage to the skin of rabbits exposed to the vapors of butyl 2-chloroethyl sulfide was assessed by conventional means, by measuring the changes in water content of tissue, accumulation of ^{125}I-labelled albumin, and by changes in the signal from a laser Doppler instrument. The content of water and ^{125}I-labelled albumin in tissue significantly increases after exposure. Extravasation of red cells, as measured by a ^{51}Cr-labelling technique, appears to be low, indicating that, under the conditions of the experiment, damage to the capillaries appears to be not very important.

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ABSTRACT

Tissue damage to the skin of rabbits exposed to the vapors of butyl 2-chloroethyl sulfide was assessed by conventional means, by measuring the changes in water content of tissue, accumulation of ^{125}I-labeled albumin, and by changes in the signal from a laser Doppler instrument. The content of water and ^{125}I-labeled albumin in tissue significantly increases after exposure. Extravasation of red cells, as measured by a ^{51}Cr-labeling technique, appears to be low, indicating that, under the conditions of the experiment, damage to the capillaries appears to be not very important.

Alternative Methods for Measuring Vesicant Agent Action on Rabbit Skin--Schmid

INTRODUCTION

The use of vesicants has long been considered an exceedingly effective means of denying territory and materials to combatants and support personnel who are not prepared to protect themselves against, or to decontaminate themselves after being exposed to, this class of chemical warfare agents. Sulfur mustard (HD) is especially insidious because skin decontamination is unlikely to be effective unless instigated within 10 to 15 minutes after the initial exposure to this vesicant (1).

Recent evidence indicates that changes in cutaneous cellular metabolism occur within a few minutes after exposure to the reputedly less toxic monofunctional sulfur mustard, n-butyl 2-chloroethyl sulfide (BCS), an analog of HD (2), although there may be no visible signs of physiological manifestations on humans for several hours, or days, after exposure to bifunctional mustard (HD). Vesicant action on animals' skin is manifested as edema and erythema, rather than as confluent blisters as seen on human skin.

It is not yet clear how the early biochemical manifestations after exposure to mustard are related to the clinical evidence, i.e., edema, erythema and skin damage, but these early metabolic changes are consistent with rapid fixation of mustard to the skin. Although the early metabolic alterations may prove useful for determining the efficacy of skin-protection formulations, classically, edema and erythema have been used to assess the irritant/injury potential of a variety of compounds, including mustard. Unfortunately, visual observation provides only semi-quantitative data, requires a highly skilled observer, and may result in significant laboratory-to-laboratory variations in the results (3-5). We therefore have undertaken the task to develop methods which will allow objective, user-independent, quantitative analyses of edema and erythema.

METHODS

Five female New Zealand rabbits, weighing approximately 3-kg, were shaven 24 hrs before the experiment. Each animal was anesthetized with one ml ketamine, injected IM, and maintained with additional injections of 0.5 ml, when needed. Five 19-mm-in-diameter glass cups containing filter paper circles impregnated with 10 µl (6.55 µmoles) neat BCS (Fig. 1a) were attached by means of double-stick discs to one side of the spine of the shaved dorsum of each anesthetized rabbit for 30 minutes (Fig. 1b). Glass cups containing filter paper, but no BCS, were attached on the opposite side of the spine as controls. At the end of 3 hrs, ^{125}I-labeled albumin and ^{51}Cr-labeled erythrocytes were injected intravenously and allowed to circulate and equilibrate for 1 hr. At the end of 4 hrs, a sample of blood was drawn; the rabbits were then euthanized with an overdose of T-61® (American Hoechst Corp.), and a large portion of the dorsal skin was removed and mounted on Saran™-wrapped cardboard to reduce shrinkage of excised skin (Fig. 1c). The circular sites of application of vesicant and the control site(s) were cut out with a motor-driven boring machine for rubber/cork stoppers equipped with a 22-mm cutting tube (Fig. 1d). Each circular tissue was then placed in a liquid scintillation uncapped vial, weighed immediately, and the vial containing the skin was placed in a vacuum oven, heated to 60°C and evacuated. The samples were removed and weighed periodically on a Mettler balance, Model 440, until a constant weight was attained. Twenty milliliters of 20% aqueous potassium hydroxide were then added to the tissue and heated at 90°C to dissolve the skin. The dpm were counted in a dual-channel gamma isotope counter to assess accumulation of ^{125}I and ^{51}Cr.

Two female 3-kg New Zealand rabbits were used to test the relative protection afforded by one layer of battle dress uniform (BDU) or Gortex® fabric against BCS. The experimental design was the same as above, except that a layer of fabric was inserted between the BCS-containing filter paper and the rabbit's skin (Fig. 2). On each of the rabbits, we used one layer of fabric per site. Five of the sites on one side were controls, five on the other side contained 10 µl of neat BCS. At the end of 3 hrs, ^{125}I-labeled albumin was injected intravenously. No ^{51}Cr-labeled erythrocytes were used in this test. Harvesting of the skin samples and analyses were performed as described in the preceding paragraph.

A laser Doppler flowmeter (Med Pacific Corp., Seattle, WA) was used to measure alterations in the skin microcirculation of two female 3-kg New Zealand rabbits after their skin was treated with BCS. The experimental procedure used was similar to that described in the first paragraph under METHODS, except no isotopes were injected into either rabbit. An extra 30 minutes, beyond the 30-minute period of exposure to BCS, were allowed to elapse to prevent contamination of the probe of the flowmeter by any BCS residue remaining on the skin surface. The animals were not sacrificed, but were kept anesthetized until the laser Doppler readings had been taken. Triplicate blood flow measurements were made for a duration of 150 seconds each time at each experimental site. The animals were kept alive and unanesthetized overnight with no apparent discomfort. They had free access to food and water. The microcirculation at the skin reaction sites and controls were again measured in triplicate the following morning after the rabbits were anesthetized with 1 ml ketamine IM. The animals were euthanized with T-61® immediately after the 24-hr readings. The laser Doppler flowmeter was calibrated and zeroed at the beginning of each experiment. A special holder was attached to the skin with double-sided sticky tape while each site was measured.

RESULTS

The mean water content of treated and untreated skin sites on the rabbits, expressed as a percentage of the total weight of the excised skin, is a measure of the amount of edema. The mean water content varied from 77.9 to 84.4 for vesicant-exposed sites and from 66.0 to 73.1 for control sites (Table 1). Each value in the table represents the mean from five sites. Although there are expected variations from animal to animal, the data clearly show an appreciably higher water content in the BCS sample than in the control sample for the five animals.

Table 1. Comparison of the Mean Water Content[1] of BCS-treated and Untreated Skin Sites of Each Rabbit

Rabbit No.	Vesicant		Control	
	Mean %[2]	S.D.[3]	Mean %[2]	S.D.[3]
1	78.0	3.12	66.0	1.58
2	77.9	1.79	70.9	1.42
3	81.3	0.32	72.9	0.87
4	80.4	1.20	72.5	1.08
5	84.4	1.68	73.1	0.58

[1]percentage of the weight of excised skin
[2]average of five sites on each animal
[3]standard deviation. N = 5

When the water content data for specific sites (vesicant and control) for the five animals were averaged (Table 2) little variation was found between different sites; however, an appreciably higher water content was found in the BCS-treated sites than in the control sites.

Table 2. Comparison of Site Variation Among Rabbits as Determined by the Mean Water Content[1] of BCS-treated and Untreated Sites

Site No.	Vesicant[2]		Control[2]	
	Mean %[3]	S.D.[4]	Mean %[3]	S.D.[4]
1	80.9	0.97	72.6	2.61
2	81.0	2.19	71.6	2.61
3	81.4	3.01	70.9	2.05
4	79.5	4.05	70.5	2.60
5	80.5	3.54	69.5	4.23

[1]percentage of weight of excised skin
[2]treated with vesicant on the left side; no treatment on the right-hand side of the spine
[3]average of five rabbits per each corresponding site
[4]standard deviation. N = 5

Deposition of ^{125}I-labelled albumin and ^{51}Cr-labelled erythrocytes, another measurement of edema and erythema, is shown for vesicant and control sites for albumin only. There were no differences in ^{51}Cr deposition, despite the presence of erythema. The ^{125}I-labelled albumin values are between 3 and 9 times higher in vesicant-treated than in control sites, with an inter-animal variation of the order of 2, from the least to the most responsive animal, when exposed to BCS (Table 3).

Table 3. Comparison of the Deposition of ^{125}I-labelled Albumin[1] at Vesicant and Control Sites of Each Rabbit

Rabbit No.	Vesicant		Control	
	Mean[2]	S.D.[3]	Mean[2]	S.D.[3]
1	731	124	96	15
2	428	98	131	10
3	444	53	72	8
4	593	133	107	20
5	990	142	106	16

[1]dpm/min/site (22-mm-diameter piece of excised skin)
[2]average of five sites on each animal
[3]standard deviation. N = 5

Visual observations of skin reactions (6) on the five rabbits were made 4 hrs post application of butyl mustard and were scored according to the Draize system as described in the footnotes of Table 4 and 5. Control sites showed a value of zero for edema and erythema.

Table 4. Evaluation of Skin Edema of Rabbits 4 Hours After Butyl Mustard Application Using Draize Scoring System[1]

Rabbit No.	Site No.				
	1	2	3	4	5
1	4	4	4	4	4
2	4	3	3	2	2
3	4	4	4	4	4
4	3	3	3	3	3
5	4	4	4	4	4

[1]1 = Very slight edema (barely perceptible)
2 = Slight edema (edges of area well defined by definite raising)
3 = Moderate edema (area raised approximately 1 mm)
4 = Severe edema (raised more than 1 mm and extending beyond area of exposure)

Table 5. Evaluation of Skin Erythema of Rabbits 4 Hours After Butyl Mustard Application Using Draize Scoring System[1]

Rabbit No.	Site No.				
	1	2	3	4	5
1	2	2	2	1	1
2	3	3	2	1	1
3	1	1	1	1	1
4	3	3	3	3	3
5	2	2	2	2	2

[1]1 = Very slight erythema
2 = Well defined erythema
3 = Moderate to severe erythema
4 = Severe erythema (beet redness) to slight eschar formation (injuries in depth)

Despite the limited data, it appears that both BDU fabric and Gortex® provide some protection against BCS vapor, as shown in Table 6.

Table 6. Effect of Fabric in Protecting Skin Against Butyl Mustard (BCS)

Site Treatment	Percent Water Content		^{125}I-labeled Albumin (dpm/site)	
	Mean[1]	S.D.[2]	Mean[1]	S.D.[2]
Control	72.0	1.4	153	98
Vesicant alone	79.9	3.2	600	205
Vesicant + BDU (0.55 mm thick)	77.4	2.8	249	36
Vesicant + Gortex® (0.45 mm thick)	75.8	2.4	169	54

[1]average of four sites (2 on each animal)
[2]standard deviation. N = 2

Cutaneous microcirculation was also measured with a laser Doppler flowmeter. Fig. 3 illustrates preliminary data from one of two experiments using two different rabbits. The output, the flow value, is given in millivolts: the larger the value, the greater the blood flow (9). The values shown are mean values from one rabbit for 5 control and 5 experimental sites. Within 30 minutes after exposure to BCS, the experimental sites display an almost twofold increase in signal, which persists for at least five hours.

DISCUSSION

The objectives of this pilot study were twofold: (a) to develop a system to quantitate measurements of edema and erythema, and (b) to provide an assay system for assessing the efficacy of formulations that protect the skin against vesicants, particularly, sulfur mustards. The rabbit was chosen as the animal model for our tests because its skin often shows a stronger response to mild

and moderate irritants than does human skin, thereby providing an additional margin of safety (6).

Since edema is the infiltration of predominantly albumin-containing fluid into the site of irritation (7), we measured both the water content of tissue and the accumulation of ^{125}I-labeled albumin. Measurements of the accumulation of ^{125}I-labeled albumin and water content of the tissues provide very sensitive indices of edema. Water content, especially, appears to reflect both intracellular and extravascular fluid accumulation.

Erythema is redness produced by congestion of the capillaries; ^{51}Cr-labeled erythrocytes were used as a measure of capillary stasis and/or damage to capillaries and extravasation of red cells. Under the conditions of our experiments, it appears that very little extravasation occurs, since the amount of ^{51}Cr at skin sites exposed to BCS is not significantly different from that of the control skin. Perhaps by using a higher radioactivity of ^{51}Cr to label the red blood cells, a small amount of activity might be detected at the BCS site; however, Issekutz (8) found that the labeling of erythrocytes with ^{51}Cr does not provide a sensitive measurement of erythema.

After the isotope experiments, erythema was measured by laser Doppler velocimetry. The preliminary data from this instrumentation (Fig. 3) suggest that it may be a useful non-invasive tool to measure changes in microvascular blood flow (9) associated with mustard damage to skin.

Negligible variation in mean water content at various sites on the rabbit skin (Table 2) indicated that, for any given rabbit, there are no large differences in response to butyl mustard, i.e., values close to the shoulder are not different from values close to the hind leg.

Variations in skin response from one rabbit to another are caused by a myriad of factors, i.e., differences in anesthesia, ambient temperature, and differences in stages of the hair growth cycle which affects capillary blood flow. Some of these variations can be minimized through careful control of experimental conditions.

We evaluated the relative protection afforded by a single thickness of battle dress uniform (BDU) fabric and Gortex® material against butyl mustard (BCS). Single layers of swatches of each cloth were placed between the cups containing vesicant and the rabbit's skin for the duration of the exposure (Fig. 2). As a positive control, the skin was exposed to vesicant with no intervening cloth. Despite limited data, it can be seen that both BDU fabric and Gortex® provide some protection against butyl mustard vapor. Conversely, these data also demonstrate that this system can be used to evaluate not only formulations, but also cloth, for their ability to protect against vesicants. These data also emphasize the value of measuring both water content of tissue and accumulation of ^{125}I-labeled albumin for quantitating edema resulting from mustard injury to animals' skin.

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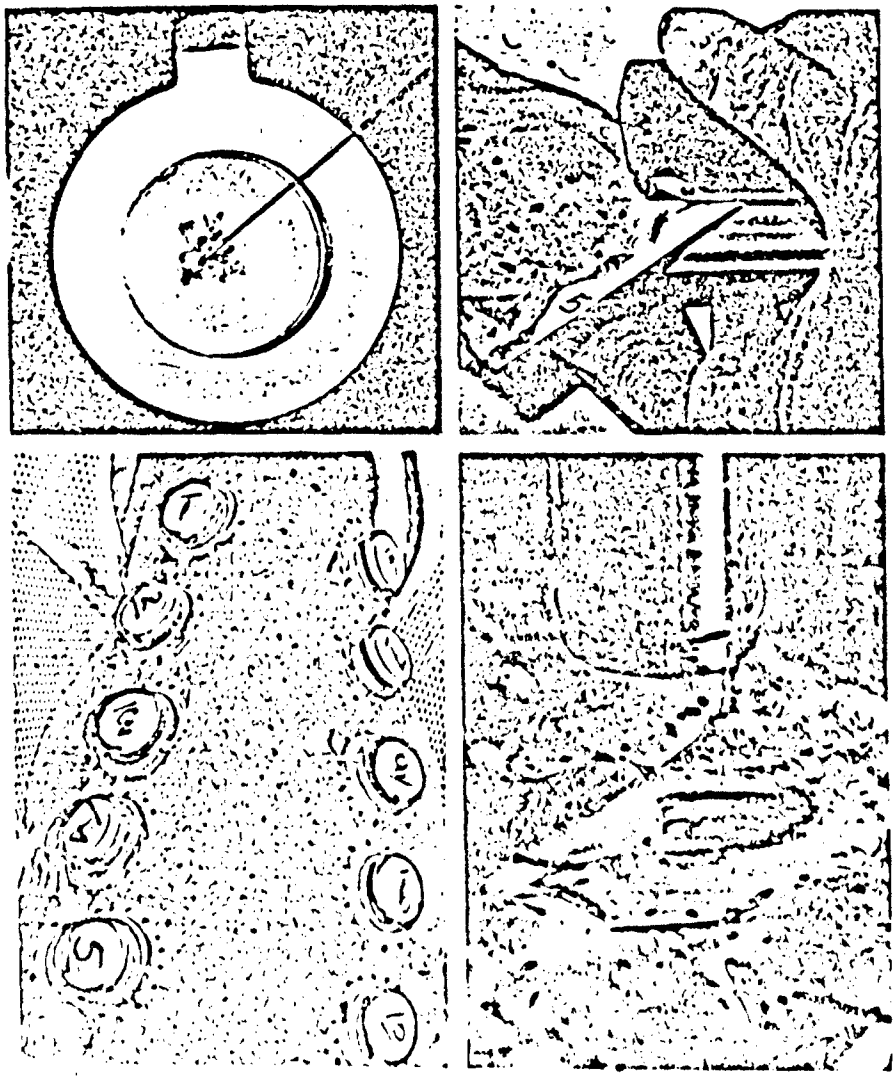

Figure 1. Glass cup containing sorbent (top left). Cups attached to dorsal aspect of an anesthetized rabbit (bottom left). Excised skin being attached to Saran-wrapped cardboard (top right). Skin sample removed with electric cork borer (bottom right).

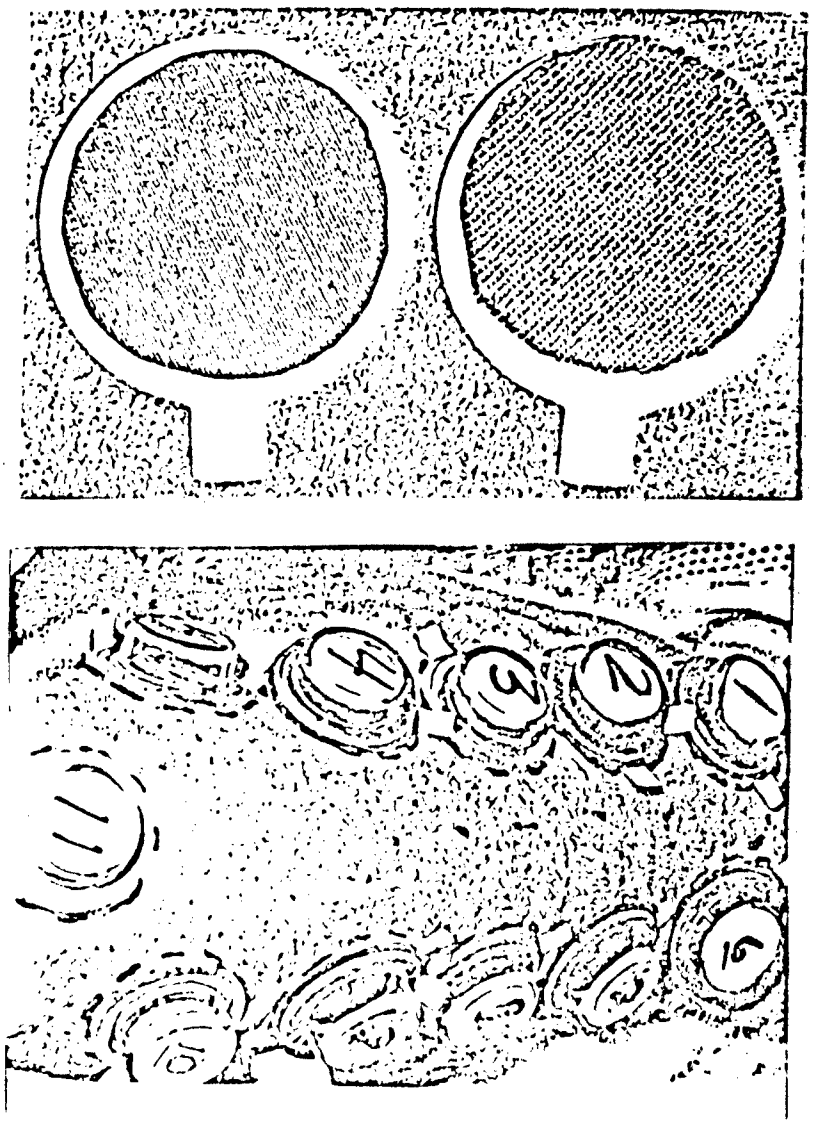

Figure 2. Top: Vesicant-containing cups with BDU (left) and Gortex® (right) swatches attached. Bottom: Vesicant-containing cups attached to the dorsal aspect of an anesthetized rabbit.

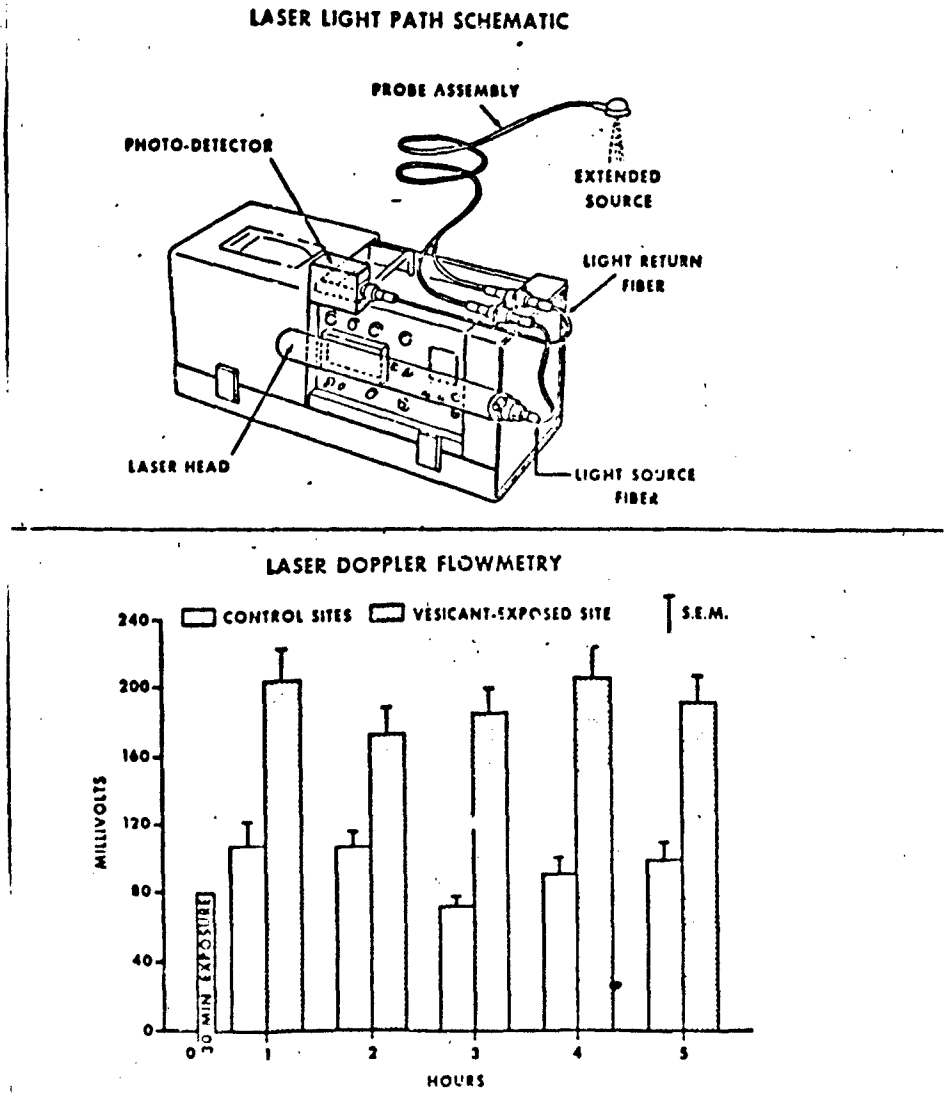

Figure 3. Top: Diagram of the laser light path. Bottom: Bar graph representing toxic reaction of rabbit skin with 5 control and 5 vesicant-exposed sites.

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